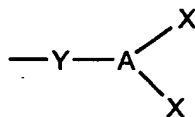


WHAT IS CLAIMED IS:

1. A conjugate comprising:
5 a biologically active agent; and
an activated, water soluble polymer comprising a polymer backbone having at least one terminus bonded to a branching moiety through a hydrolytically stable linkage, the branching moiety being covalently bonded to at least two reactive groups, each reactive group comprising a reactive moiety,
10 said biologically active agent being covalently linked to at least one of said reactive groups.
2. The conjugate of Claim 1, wherein said biologically active agent is selected from the group consisting of proteins, peptides, lipids, polysaccharides, nucleotides,
15 and derivatives thereof.
3. The conjugate of Claim 1, wherein said biologically active agent has a free amine or thiol group and at least one of said reactive groups comprises an amine-reactive or thiol-reactive moiety.
20
4. The conjugate of Claim 1, wherein the branching moiety comprises a carbon atom.
5. The conjugate of Claim 1, wherein said terminus of the backbone is bonded
25 to the structure:



wherein Y is a hydrolytically stable linkage, A is a branching moiety, and X and X', which may be the same or different, are reactive groups comprising a reactive
30 moiety.

6. The conjugate of Claim 5, wherein A is CH.

7. The conjugate of Claim 5, wherein at least one of said X and X' has the
35 structure -W-Z, wherein W is a tethering group and Z is said reactive moiety.

8. The conjugate of Claim 7, wherein W is selected from the group consisting
of alkyl chains, ether chains, ester chains, amide chains, and combinations thereof.

40 9. The conjugate of Claim 7, wherein W is selected from the group consisting
of $-(CH_2)_m-$, $-(CH_2)_m-O-$, $-O-(CH_2)_m-$, $-(CH_2)_m-O_2C-CH_2CH_2-$, and $-(CH_2)_m-O-$
 $(CH_2)_r-$, wherein m and r are independently 1-10.

10. The conjugate of Claim 7, wherein Z is selected from the group consisting
45 of active esters, active carbonates, aldehydes, isocyanates, isothiocyanates, epoxides,
alcohols, maleimides, vinylsulfones, hydrazides, dithiopyridines, and iodoacetamides.

11. The conjugate of Claim 7, wherein Z is selected from the group consisting
of -OH, -CO₂H, -CHO, -SO₂-CH=CH₂, and -CO₂-Q, wherein Q is N-succinimidyl,
50 sulfo-N-succinimidyl, or 1-benzotriazolyl.

12. The conjugate of Claim 1, wherein said reactive moiety is selected from
the group consisting of active esters, active carbonates, aldehydes, isocyanates,
isothiocyanates, epoxides, alcohols, maleimides, vinylsulfones, hydrazides,
55 dithiopyridines, and iodoacetamides.

13. The conjugate of Claim 1, wherein said reactive moiety is selected from
the group consisting of -OH, -CO₂H, -CHO, -SO₂-CH=CH₂, and -CO₂-Q, wherein
Q is N-succinimidyl, sulfo-N-succinimidyl, or 1-benzotriazolyl.

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14. The conjugate of Claim 1, wherein said polymer backbone is selected
from the group consisting of linear or branched poly(alkylene oxide), linear or

branched poly(vinyl pyrrolidone), linear or branched poly(vinyl alcohol), linear or branched polyoxazoline, and linear or branched poly(acryloylmorpholine).

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15. The conjugate of Claim 1, wherein said polymer backbone has a molecular weight of about 100 to about 100,000 Da.

16. The conjugate of Claim 15, wherein said polymer backbone has a
70 molecular weight of about 6,000 to about 80,000 Da.

17. The conjugate of Claim 1, wherein said polymer backbone is a
poly(alkylene oxide) selected from the group consisting of poly(ethylene glycol),
poly(propylene glycol), and copolymers of ethylene glycol and propylene glycol.
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18. The conjugate of Claim 1, wherein said polymer backbone is
poly(ethylene glycol) having a molecular weight of about 200 to about 100,000 Da.

19. The conjugate of Claim 1, wherein the hydrolytically stable linkage is
80 selected from the group consisting of -O-, -S- and -CO-NH-.

20. The conjugate of Claim 1, wherein the at least two reactive groups have
the structure -W-Z and -W'-Z', respectively, wherein Z and Z' are said reactive
moieties, and W and W' are tethering groups.
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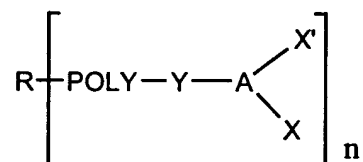
21. The conjugate of Claim 20, wherein W and W' are selected from the group
consisting of alkyl chains, ether chains, ester chains, amide chains, and combinations
thereof.

22. The conjugate of Claim 20, wherein W and W' are independently selected
90 from the group consisting of $-(CH_2)_m-$, $-(CH_2)_m-O-$, $-O-(CH_2)_m-$, $-(CH_2)_m-O_2C-$,
 CH_2CH_2- , and $-(CH_2)_m-O-(CH_2)_r-$, wherein m and r are independently 1-10.

23. The conjugate of Claim 20, wherein Z and Z' are each independently
95 selected from the group consisting of active esters, active carbonates, aldehydes,
isocyanates, isothiocyanates, epoxides, alcohols, maleimides, vinylsulfones,
hydrazides, dithiopyridines, and iodoacetamides.

24. The conjugate of Claim 20, wherein Z and Z' are each independently
100 selected from the group consisting of -OH, -CO₂H, -CHO, -SO₂-CH=CH₂, and -CO₂-
Q, wherein Q is N-succinimidyl, sulfo-N-succinimidyl, or 1-benzotriazolyl.

25. The conjugate of Claim 1, wherein said polymer has the structure:



105

wherein

R is a central core;

POLY is a polymer backbone;

A is a branching atom;

110 Y is a hydrolytically stable linkage;

n is from 2 to 200; and

X and X' are reactive groups comprising a reactive moiety.

26. The conjugate of Claim 25, wherein at least one of said X and X' has the
115 structure -W-Z, wherein W is a tethering group and Z is said reactive moiety.

27. The conjugate of Claim 26, wherein W is selected from the group
consisting of alkyl chains, ether chains, ester chains, amide chains, and combinations
thereof.

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28. The conjugate of Claim 26, wherein W is selected from the group
consisting of -(CH₂)_m -, -(CH₂)_m-O-, -O-(CH₂)_m-, -(CH₂)_m-O₂C-CH₂CH₂-, and -

$(\text{CH}_2)_m\text{-O-(CH}_2)_r$, wherein m and r are independently 1-10.

125 29. The conjugate of Claim 26, wherein Z is selected from the group consisting of active esters, active carbonates, aldehydes, isocyanates, isothiocyanates, epoxides, alcohols, maleimides, vinylsulfones, hydrazides, dithiopyridines, and iodoacetamides.

130 30. The conjugate of Claim 26, wherein Z is selected from the group consisting of -OH, -CO₂H, -CHO, -SO₂-CH=CH₂, and -CO₂-Q, wherein Q is N-succinimidyl, sulfo-N-succinimidyl, or 1-benzotriazolyl.

 31. The conjugate of Claim 25, wherein each POLY is selected
135 from the group consisting of linear or branched poly(alkylene oxide), linear or branched poly(vinyl pyrrolidone), linear or branched poly(vinyl alcohol), linear or branched polyoxazoline, and linear or branched poly(acryloylmorpholine).

 32. The conjugate of Claim 25, wherein each POLY has a molecular weight of
140 about 100 to about 100,000 Da.

 33. The conjugate of Claim 32, wherein each POLY has a molecular weight of about 6,000 to about 80,000 Da.

145 34. The conjugate of Claim 25, wherein each POLY is a poly(alkylene oxide) selected from the group consisting of poly(ethylene glycol), poly(propylene glycol), and copolymers of ethylene glycol and propylene glycol.

 35. The conjugate of Claim 34, wherein each POLY is poly(ethylene glycol)
150 having a molecular weight of about 200 to about 100,000 Da.

 36. The conjugate of Claim 25, wherein R is a moiety selected from the group consisting of lysine, glycerol, pentaerythritol, and sorbitol.

155 37. The conjugate of Claim 25, wherein each Y is selected from the group consisting of -O-, -S- and -CO-NH-.

 38. The conjugate of Claim 25, wherein X and X' are -W-Z and -W'-Z', respectively, wherein Z and Z' are said reactive moieties, and W and W' are tethering
160 groups.

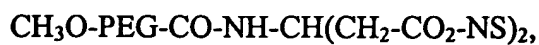
 39. The conjugate of Claim 38, wherein W and W' are selected from the group consisting of alkyl chains, ether chains, ester chains, amide chains, and combinations thereof.

165 40. The conjugate of Claim 38, wherein W and W' are independently selected from the group consisting of $-(CH_2)_m-$, $-(CH_2)_m-O-$, $-O-(CH_2)_m-$, $-(CH_2)_m-O_2C-$, CH_2CH_2- , and $-(CH_2)_m-O-(CH_2)_r-$, wherein m and r are independently 1-10.

170 41. The conjugate of Claim 38, wherein Z and Z' are each independently selected from the group consisting of active esters, active carbonates, aldehydes, isocyanates, isothiocyanates, epoxides, alcohols, maleimides, vinylsulfones, hydrazides, dithiopyridines, and iodoacetamides.

175 42. The conjugate of Claim 38, wherein Z and Z' are each independently selected from the group consisting of -OH, -CO₂H, -CHO, -SO₂-CH=CH₂, and -CO₂-Q, wherein Q is N-succinimidyl, sulfo-N-succinimidyl, or 1-benzotriazolyl.

 43. The conjugate of Claim 1, wherein the polymer has a formula selected
180 from the group consisting of:



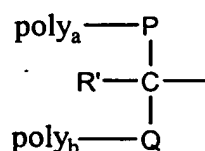
185 CH₃O-PEG-O-CH(CH₂-O₂C-CH₂CH₂-CO₂-NS)₂,
 (OHC-CH₂CH₂-O-CH₂)₂-CH-NHCO-PEG-CONH-CH(OCH₂CH₂-CHO)₂,
 PEG-[CONH-CH(OCH₂CH₂-CHO)]₄,
 CH₃O-PEG-CO-NH-CH(CO₂-NS)[CH₂CH₂CO₂-NS],
 CH₃O-PEG-O-CH₂CH₂CH(CO₂H)₂,
 190 CH₃O-PEG-O-CH₂CH₂CH(CH₂OH)₂, and
 CH₃O-PEG-O₂CNHCH(CH₂CO₂H)₂, wherein NS is N-succinimidyl.

44. The conjugate of Claim 1, wherein the polymer backbone is branched
 poly(ethylene glycol) and the reactive groups are selected from the group consisting of
 195 active esters, active carbonates, aldehydes, isocyanates, isothiocyanates, epoxides,
 alcohols, maleimides, vinylsulfones, hydrazides, dithiopyridines, and iodoacetamides.

45. The conjugate of Claim 1, wherein the polymer backbone comprises
 methoxy poly(ethylene glycol) disubstituted lysine.

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46. The conjugate of Claim 1, wherein the polymer backbone has the
 structure:



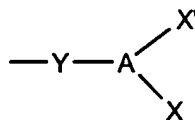
wherein

205 poly_a and poly_b are water-soluble and non-peptidic polymer backbones;
 R' is a nonreactive moiety; and
 P and Q are nonreactive linkages.

47. The conjugate of Claim 46, wherein poly_a and poly_b are both methoxy
 210 poly(ethylene glycol).

48. The conjugate of Claim 46, wherein R' is H, methyl or a water-soluble and
 non-peptidic polymer backbone.

215 49. The conjugate of Claim 46, wherein the polymer backbone is bonded to
the structure:



220 wherein Y is a hydrolytically stable linkage, A is a branching moiety, and X
and X', which may be the same or different, are reactive groups comprising a reactive
moiety.

50. The conjugate of Claim 49, wherein A is CH.

225 51. The conjugate of Claim 49, wherein at least one of said X and X' has the
structure -W-Z, wherein W is a tethering group and Z is said reactive moiety.

230 52. The conjugate of Claim 51, wherein X and X' have the structure -W-Z and
-W'-Z', respectively, wherein Z and Z' are said reactive moieties, and W and W' are
tethering groups.

235 53. The conjugate of Claim 52, wherein Z and Z' are each independently
selected from the group consisting of active esters, active carbonates, aldehydes,
isocyanates, isothiocyanates, epoxides, alcohols, maleimides, vinylsulfones,
hydrazides, dithiopyridines, and iodoacetamides.